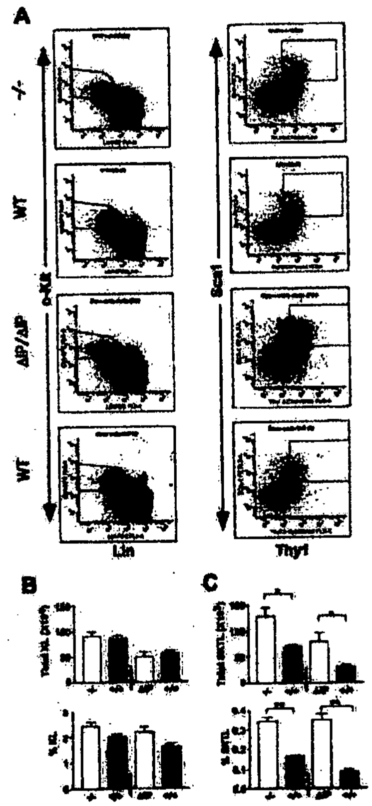


Fig. 1. Increased HSC numbers in SHIP-/- and SHIPΔ IP/ΔIP mice. (A) FACS analysis of SKTL HSC cells and KL stem/progenitors in SHIP-/-, SHIPΔ IP/Δ IP and their WT littermates. (B) Absolute and re numbers of KL stem/progenitors and (C) SKTL HSC in SHIP-/-, SHIPΔ IP/Δ IP and their WT litter The values determined for SHIP-/- and SHIPΔ IP/Δ IP mice that are significantly different from the matched WT counterparts are indicated by the following symbols: *, p<0.05 and **, p<0.0001. Bone mar row cells were obtained by flushing two intact femurs and two tibias from each mouse. Cells were stained and then analyzed using a FACS Vantage (Becton Dickinson) for the presence of HSC as defined by the Sca1-Thy1.2-ckit-Lin (SKTL) HSC phenotype. There was no significant difference observed in the absolute or relative number KL stem/progenitor cells present in SHIP mutant strains and their WT counterparts. Note that the background of SHIP-/- mice is essentially C57BL6/J while the SHIPΔ IP/Δ IP are F2(12 C57BL6/J). Thus, the SHIP mutation impacts HSC frequency despite differences in genetic background, further attesting to its role in regulating HSC numbers.

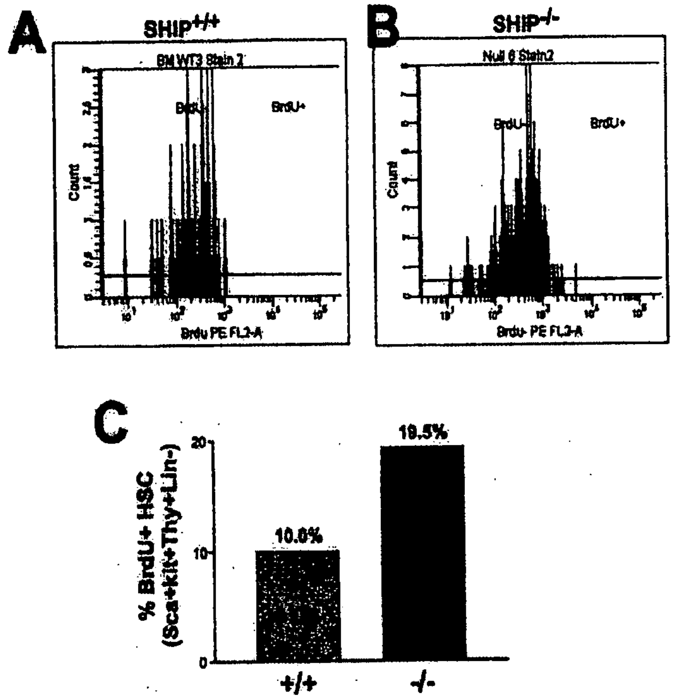


Figure 2. A greater proportion of SHIP-deficient HSC are actively proliferating. BrdU staining in Sca+Thy1+kit+Lin- HSC from a representative SHIP+/+ (A) and SHIP-/- (B) mouse. C. Mean BrdU incorporation in Sca+Thy1+kit+Lin- HSC following a 9 day BrdU pulse of SHIP-/- and SHIP+/+ mice (n=2 per genotype). The percentage of HSC positive for BrdU incorporation was determined by comparison with an isotype con trol Ab conjugated to PE (Pharmingen). Only HSC with BRdU staining above that seen in the isotype control were considered positive for BrdU incorporation and this fraction was used to determine the percent BrdU positive HSC. Mice were placed on drinking water containing bromodeoxyuridine (BrdU) at 1mg/ml for 9 days. The mice were then sacrificed and bone marrow cells were isolated from intact tibia and femurs. The BM cells were initially stained with the Lin panel-FITC, c-Kit-APC, Sca1-PE-Cy7 and Thy1.2-Cychrome (eBioscience) antibodies. Following cell permeabilization, the sam ples were stained with anti-BrdU-PE (BD Pharmingen) and analyzed on a FACS Vantage/DIVA.

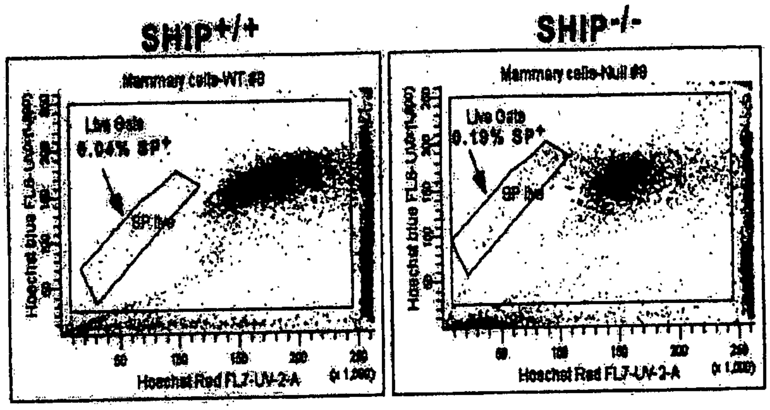


Fig. 3. Mammary gland stem cells (MSC) as identified by the SP phenotype (Side Population) based on exclusion of Hoechst dye. Mammary glands were prepared from adult SHIP+/+ and SHIP-/- littermates and a single cell suspension was prepared. Viable cells were analyzed for exclusion of the Hoechst dye and 7AAD. The percentage of MSC present in the mammary gland of each genotype is indicated. Note that there is almost a five-fold increase in the frequency of MSC present in the SHIP-/- mammary glands.

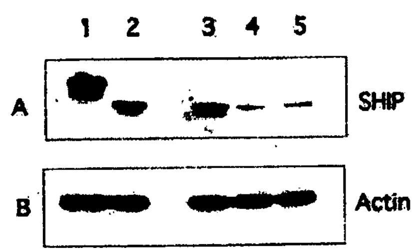


Fig. 4. Method to render primary stem cells SHIP deficient by RNA interference. Embryonic stem cells that express the SHIP gene were transfected with an irrelevant shRNA vector (Lane 3) or with two different SHIP-specific shRNA vectors (Lanes 4 and 5). The cells were then lysed and equal quantities of whole cell extracts were blotted with either anti-SHIP (Panel A) or anti-Actin (Panel B). Lane 2- Untreated ES cells. Lane 1 - Untreated RAW264.7 cells that express the SH2 containing SHIP p135 and p145 isoforms. Panel A shows significant reduction of SHIP expression in primary ES cells after transfection of SHIP-specific shRNA vectors in the absence of selection. Please note that these vectors will also interfere with the larger SH2-containing isoforms expressed in differentiated hematopoietic cells.

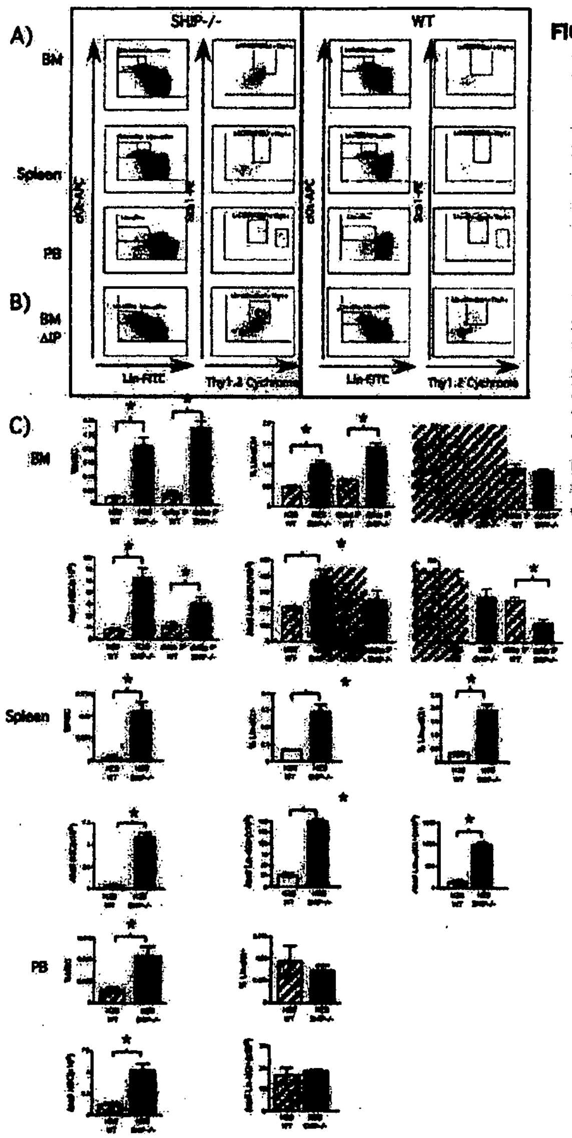


FIG. 6 A) Bone marrow (BM), spleen, and peripheral blood (PB) from SHIP-/- H2B mice were analyzed for the presence of hematopoietic stem cells (HSC;KTLS:Lin-cKit+ Sca1+ Thy1+) and early progenitor cells (Lin-cKit+) and late progenitor cells (Lin^{low}cKit+) by flow cytometry analysis. B) Bone marrow from $SHIP^{\Delta IP/\Delta IP}$ was analyzed for HSC and progenitor cells as mentioned for BM H2B. C) Statistical analysis showing the relative and absolute numbers of HSC and progenitor cells different hematopoietic organs. The statistical analysis was performed using the unpaired Student T test. Results were considered significant (indicated by an *) when p values were under 0.05.

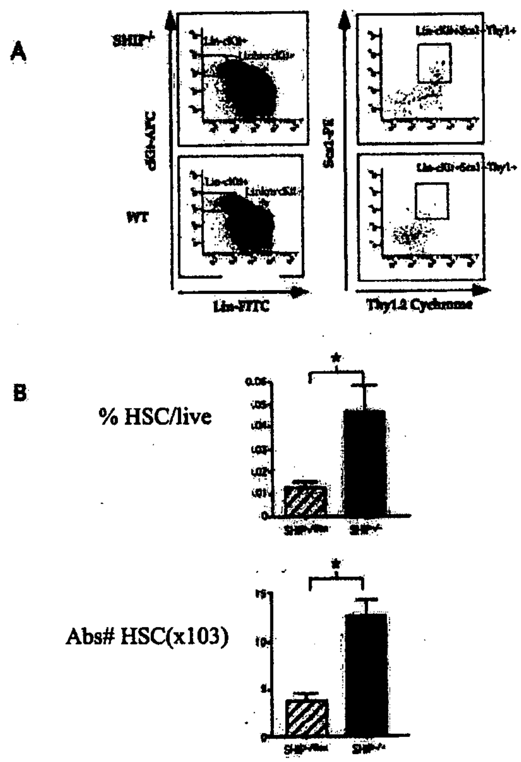


FIG. 6 HSC;KTLS (Lin-cKit+Sca1+Thy1+) cells are increased in mice in which SHIP was ablated during adulthood. A) Bone marrow (BM) from Mx-CRE SHIP$^{-/-}$ and SHIP$^{+/-}$ mice were analyzed for the presence of hematopoietic stem cells (HSC; Lin-cKit+Sca1+Thy1+) by flow cytometry analysis. Mx-CRE SHIP$^{-/-}$ mice are created by injecting MxCRE SHIPFlox/- mice with polyIC, which induces the production of interferon. Interferon acts on the Mx promoter to cause the transcription of CRE. CRE will then cause recombination of the flox site, leading to SHIP deletion. This model allow use to study the effect of SHIP removal during adulthood. B) Statistical analysis showing the relative numbers of HSC from Mx-CRE SHIP-/- and SHIP-/flox. The statistical analysis was performed using the unpaired Student t test in Prism 4. Results were considered significant when $p<0.005$. *$p<0.05$.

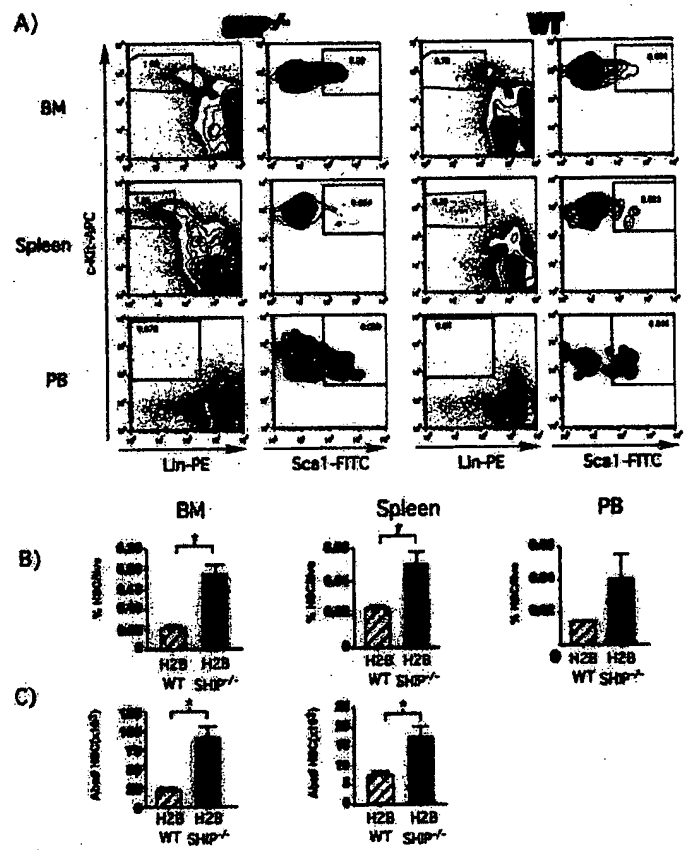


FIG. 7 The same group that defined the KTLS phenotype, found another method to isolate immunophenotype to isolate HSC, which is Lin-Flk2-cKit+Sca1+(LFKS). LFKS cells are also increased in the SHIP$^{-/-}$ mice. A) Bone marrow (BM), spleen, and peripheral blood (PB) from SHIP$^{-/-}$ H2B mice were analyzed for the presence of hematopoietic stem cells following a different immunophenotype (HSC;Lin-Flk2-cKit+Sca1+) by flow cytometry analysis, on FacsCalibur. B) Statistical analysis showing the relative numbers of HSC in the different hematopoietic organs. C) Statistical analysis showing the absolute numbers of HSC in the different hematopoietic organs. The statistical analysis was performed using the unpaired Student T test. Results were considered significant (indicated by an *) when p values were under 0.05.

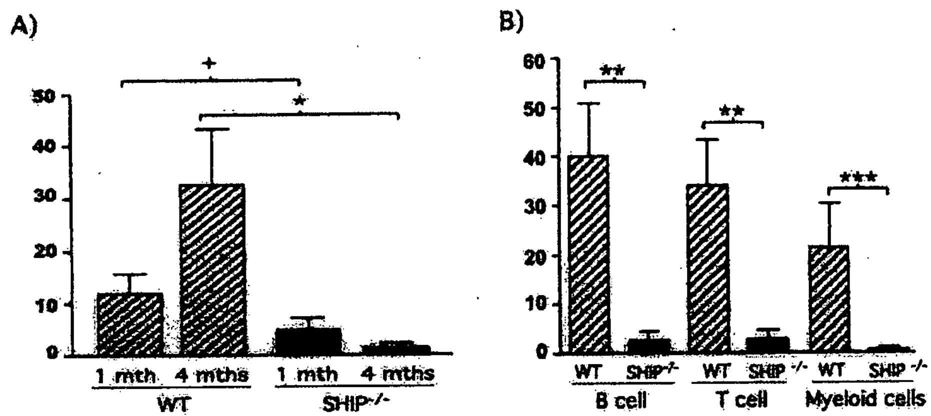


FIG. 8 Level of reconstitution after direct competition assay. Direct competition assay was performed by sorting KTLS (Lin-cKit+Sca1+Thy1+) cells from SHIP$^{-/-}$ Ly5.2 mice and from WT Ly5.1 mice. 200 SHIP$^{-/-}$ Ly5.2 and 200 WT Ly5.1 KTLS were then injected into the same animal with 40 000 Sca1^{-} supporting cells (Ly5.1/Ly5.2). The recipient mice were Ly5.1/Ly5.2, which allow us to identify which cells in the immune system comes from the recipient, WT donor or SHIP$^{-/-}$ donor. The recipient mice had undergone total body irradiation (dose: 600 rads and 2 hours later 400 rads) 2 hours before being transplanted with the test cells. After transplantation the mice were given antibiotic water to prevent opportunistic pathogen. At different time point after transplantation, the mice were tested for reconstitution of the hematopoietic compartment. A) We show global reconstitution 4 weeks and 4 month after transplantation. The result show no signficant difference 4 weeks after transplantation between the WT and the SHIP-/- HSC ability to reconstitute the hematopoietic system. Four 4 months after transplantation, the proportion of hematopoietic cells derived from the WT KTLS is significantly higher than the one derived from SHIP$^{-/-}$ KTLS cells . B) We shows the level of reconstitution, 4 months after transplantation, in 3 different hematopoietic lineage to show that the cells are pluripotent. Again the level of reconstitution from the WT KTLS is significantly higher than from SHIP$^{-/-}$ KTLS. This is the result of 11 mice done in two different experiments. Statistical significance was established using Prism 4 software, unpaired student t test. + $p>0.05$, * $p<0.01$, ** $p<0.005$, *** $p<0.05$.

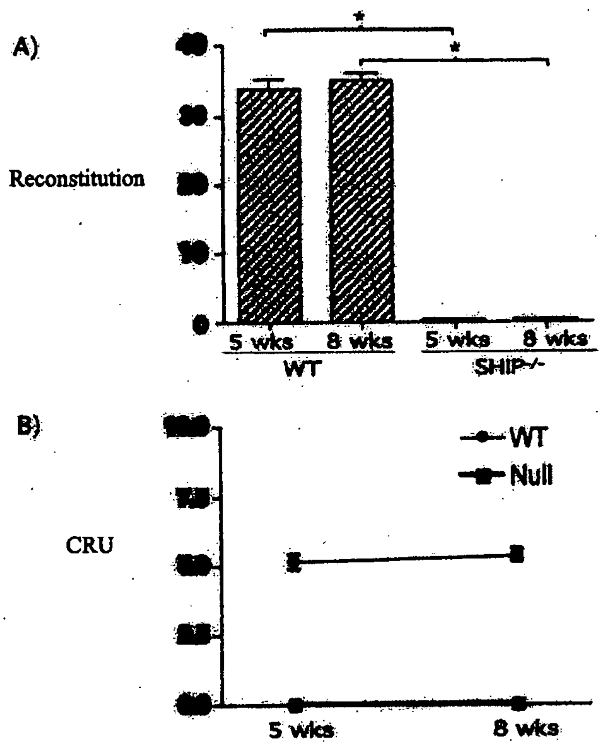


FIG. 9 Competitive repopulation assay shows that SHIP-/- bone marrow has less CRU than WT litternmates BM. Mice transplanted: Ly5.1 C57Bl6 mice that were irradiated with one-single dose of 950 rads. The tested cells were Ly5.2 cells from either SHIP-/- mice and WT mice and the competing cells were Ly5.1. A) Level of reconstitution of the hematopoietic system by the tested donor BM. WT BM level of reconstitution is significantly higher than for SHIP-/- BM. Student t test $p<0.0001$. B) Number of competitive repopulation unit (CRU) was established following a method developed by D.E. Harrison, where donor CRU= (10x%donor)/(100-%donor). 10 is the number of of CRU present in the competing BM (Ly5.1). WT BM has a significantly higher number of CRU compared to SHIP-/- mice. Unpaired t test $p=0.0005$.

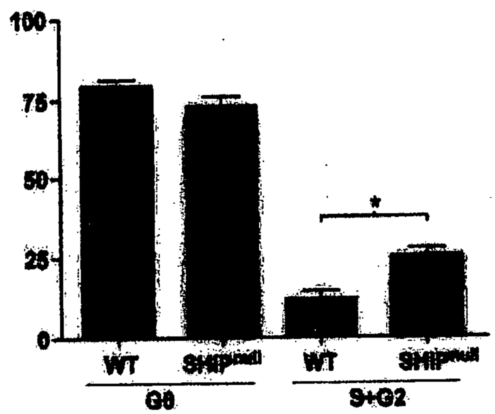
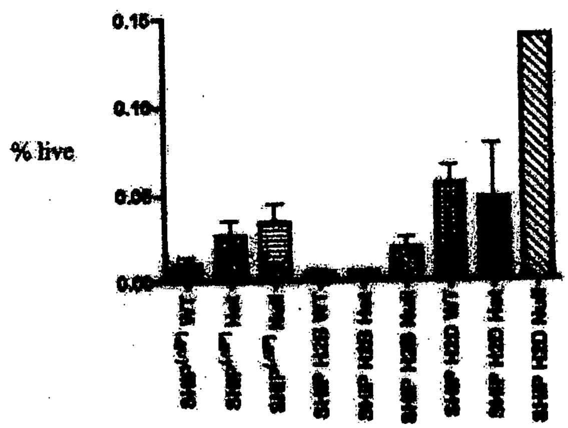


FIG. 16 Cell cycle analysis on BM from SHIP deficient and WT mice revealed that a greater proportion of $SHIP^{-/-}$ HSC in cell cycle. Bar graph representing the proportion of $Lin^{-}cKit^{+}Sca1^{+}$ cells that are G0 (resting) or in S/G2 phase (dividing). This graph includes results from experiment performed using $SHIP^{-/-}$ mice on a C57BI6 background and $SHIP^{\Delta IP/\Delta IP}$ on a 129SvJ background with respective WT counterparts.

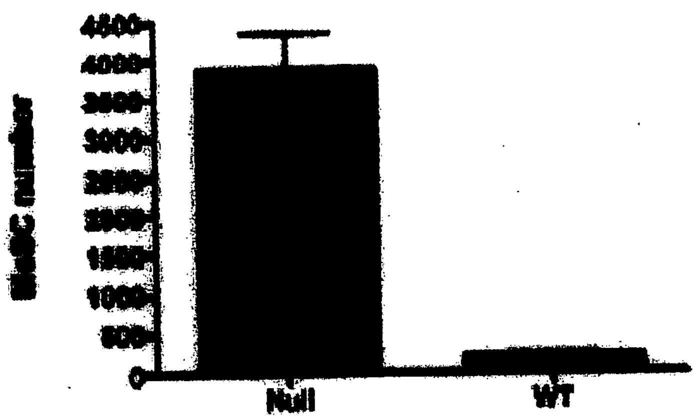
SHIP deficiency alters the size of the Mammary stem cell compartment



Mammary stem cells were analyzed from both H2B and H2D SHIP mice and from Æ IP (Rock) mice. Cells were isolated from the third and fourth mammary glands of 6-8 week old female mice. The glands were made into a single cell suspension through physical processing and enzymatic digestion. The mammary gland single cell suspension was examined for MaSC using the lineage negative gate of CD45-, and two positive gates SP+ and Sca+ (See Welm et al 2002).

FIG. 11

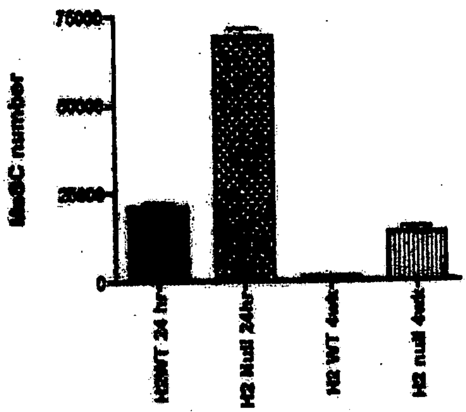
SHIP deficiency leads to an increase in mesenchymal stem cell number



MeSC were analyzed from SHIP+/+ and SHIP-/- mice on a C57Bl6/J ackground. Femurs and tibiae were collected from three sets of mice. The muscle, cartilage, and marrow were removed. The bone was crushed with a mortar and pestle and the fragments were digested with collagenase. Cells were plated with MeSC isolation media in triplicate at equal density. They were allowed to attach for 24 hours at the conclusion of which the non-adherent cells were washed away and the adherent cells were counted (See Pelster et al 2003). A students t-test was performed and the P-value < 0.0001.

FIG. 12

Analysis of SHIP+/+ and SHIP-/- MeSC



Bar graphs represent the number of MeSC per 1 million input whole bone marrow cells plated at time zero. Cells were counted, after lifting with trypsin, at 24 hours and 4 weeks post time zero using a hematocytometer. Total cell numbers decreased at 4 weeks as the cultures became a more homogenous population, though the difference that exists between the SHIP+/+ and SHIP-/- cell counts at each time point increased from a factor of 3.2 for the WT to 11.7 for the null which has a p value of $p < 0.003$ indicating that this increase is significant. * $p < 0.0001$ ** $p < 0.01$ by a two-tailed students t-test.

FIG. 13